Boll. Ist. Ent. "G. Grandi" Univ. Bologna, 48: 1-10, 1993.

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Artificial culture of the parasitoid *Exorista larvarum* L. (Dipt. Tachinidae) on oligidic media: improvements of techniques.(*)([1])([2])

Introduction

In a previous paper (Mellini *et al.*, 1993) we described the excellent results obtained by culturing *Exorista larvarum* L. on an artificial diet based on bovine serum (75%) and extract of *Galleria mellonella* L. pupae (20%). In practice, we did not observe any noteworthy difference between rearing this parasitoid on the above medium or on the factitious host *G. mellonella*. *In vitro* and *in vivo*, pupal and adult yields were found to be almost identical. Puparium weights and development times were also similar. Adults were fecund and females readily oviposited on *G. mellonella* larvae. The eggs were viable and produced a normal second generation within the host.

Success by rearing *E. larvarum in vitro* has been obtained also by Bratti and Coulibaly (in press) on tissue culture-based diets.

The artificial culture of *E. larvarum* on the diet developed by us represents one of the most successful attempts so far made at rearing parasitoids *in vitro*, not only with regard to Tachinidae, but also to Hymenoptera Terebrantia. It should be noted that most studies on *in vitro* rearing concern parasitoids belonging to the latter group. In particular, success has been obtained with oophages of the genus *Trichogramma* (Westw.).

The diet employed by us for *E. larvarum* was already quite inexpensive and the rearing technique relatively simple. Notwithstanding, modifications were made in order to improve artificial culture efficiency and reduce cost so as to permit the mass-rearing of this parasitoid.

E. larvarum is a well-known larval parasitoid of many Lepidoptera, including serious foliage-eating pests of broadleaf trees such as *Lymantria dispar* L. and *Hyphantria cunea* (Drury), against which this tachinid can be utilized in IPM pro-

(*) Accepted for publication September 1, 1993.

([1]) Studies on Diptera Tachinidae. Fifty-seventh contribution.

([2]) Research supported by the Project of the Italian Ministry of Agriculture and Forestry (MAF) «Lotta biologica e integrata per la difesa delle piante agrarie e forestali».

grammes. Our research group, in co-operation with American scientists, is already developing a project on this topic.

E. larvarum appears to be suitable for mass-production on artificial diets mainly because of the following reasons.

a) The larvae exhibit a relatively simple behaviour, i.e. they form an integumental primary respiratory funnel and substantially develop independently of host physiology. As a consequence, once the host has been attacked, the parasitoid grows quite rapidly.

b) The larvae behave gregariously and even numerous groups may therefore be reared in a single container.

c) The parasitoid is quite resistant to the moulds and bacteria which may contaminate the artificial diet in spite of care being taken to maintain asepsis.

Materials and methods

All *E. larvarum* individuals employed for the present and the previous study (Mellini *et al.* 1993) were obtained from a stock colony continuously reared in our laboratory using *G. mellonella* as a factitious host. The latter proved to be well suited to this tachinid, as it is for many other parasitoids.

The colony was established in 1992 from a few *E. larvarum* adults reared from larvae of *Hyphantria cunea* which had been collected in the province of Bologna.

Most of the techniques utilized in the present study have been described in the previous paper. Parasitoid macrotype eggs were removed from superparasitized *G. mellonella* last-instar larvae and placed on the media within 1-2 hours following oviposition. Pupa extract, also obtained from *G. mellonella*, was added to bovine serum in the same ratios as in the previous study.

The artificial culture of *E. larvarum* larvae was carried out at 27-30°C. Rearing containers were individually wrapped in tinfoil so as to ensure complete darkness, except when they were removed for visual inspection which was performed every day.

In the present study, the following modifications to the previously employed technique were examined.

I. Replacement of plastic multiwell plates with glass Petri dishes.

Given that *E. larvarum* larvae behave gregariously *in vitro* as they do *in vivo*, we considered the possibility of rearing them in groups in glass Petri dishes rather than individually in plastic multiwell plates. In this way, the artificial rearing of this tachinid could be less expensive as glass Petri dishes can be sterilized by autoclaving and, therefore, re-utilized, whereas plastic plates are only for single use.

Five-cm diameter glass Petri dishes containing 15 g of diet and 24 eggs (treatment A) were compared to plastic multiwell plates containing the same total amount of diet (0.62 g X 24 wells) and the same number of eggs placed individually into the wells (treatment B). In addition to a difference in crowding patterns, the pabulum surface was also found to vary in the two treatments, being

equal to 20 cm^2 in Petri dishes and to a total of 48 cm^2 in multiwell plates. In treatment A, therefore, diet thickness was more than twice that in treatment B.

II. Replacement of agar with absorbent cotton, used as a physical support for the liquid medium.

Besides being very expensive, agar requires careful preparation in order to achieve adequate gelation and distribution of the liquid diet in rearing containers. Moreover, being an indigestible material, it is inappropriate for tachinid larvae, in which defecation does not occur until late in the larval stage. By contrast, carefully sterilized absorbent cotton is a very inexpensive and easy-to use support, especially in the Petri dishes utilized for mass-rearing.

The liquid diet was pipetted onto sterilized cotton, which had been previously placed in Petri dishes. The diet was immediately absorbed by the cotton.

Sterilization was performed following two different procedures. At first, the Petri dishes containing cotton were individually wrapped in tinfoil (in order to prevent cotton from coming into contact with vapour) and autoclaved at 130°C for 15 minutes. This procedure, however, proved to be inadequate as diet-soaked cotton was found to become easily contaminated by moulds and bacteria. In subsequent tests, therefore, sterilization was performed in a dry-stove at 250°C and more satisfactory results were obtained.

III. Replacement of pupa extract with pupa homogenate.

Tests were performed in multiwell plates with diets set in agar. Pupa homogenate was obtained by squeezing pupae in a syringe. The large pieces of cuticle were then removed and the homogenate was sterilized by autoclaving at 130°C for 15 minutes. Pupa extract preparation required much additional work. Non-sterilized pupa homogenate had to be centrifuged and suspended compounds removed. The extract was then sterilized by passing it through a 0.45μ millipore filter.

IV. Amount of diet for each larva.

Experiments were performed in Petri dishes only. Groups of 24, 48, 72 and 96 eggs were put in Petri dishes, each containing 15 g of diet. The average quantity of diet per larva was therefore to be of 625, 313, 208 and 156 mg, respectively.

As host-derived material, pupa extract was used in the first tests and pupa homogenate in subsequent ones.

V. Attempts to reduce host-derived material in the diet.

Host-derived material is the most expensive diet ingredient as it requires the host to be available in the first place together with more or less complex procedures for its extraction and preparation. Experiments were performed, therefore, in order to determine to what extent it could be reduced in the diet without affecting the qualitative and quantitative characteristics of the parasitoid production.

Five Petri dishes, each containing the standard total amount of diet (15 g), were

used for the 5 treatments. The percentage of host-derived material was progressively reduced by half in each of the first 4 treatments, i.e. from 20 to 10 to 5 and to 2.5, respectively, and to 0 in the last. Deleted host material was replaced with an equal amount of bovine serum, the percentage of which for the 5 treatments was 75, 85, 90, 92.5 and 95, respectively. Twenty-four eggs were placed in each dish. Both pupa extract and homogenate were utilized in this experiment.

Whenever contamination occurred, moulds and bacteria were often found to spread more and more rapidly over the diet surface. The harmful effects of contamination on parasitoid development varied depending on the time of contamination onset. If early, puparium yields dropped, almost reaching zero. If late, puparium yields dropped to lower levels than on non-contaminated diets and, in any case, undersized puparia formed, which often failed to let the adult emerge.

As a rule, puparia were weighed within 2 days of formation. As is well-known, as time passes, tachinid puparia undergo a progressive weight loss, which was found to be as high as 10% in *Pseudogonia rufifrons* Wied. (Campadelli 1980).

After weighing, the puparia were transferred to empty Petri dishes. Their permanence in containers with diet remains at a very high humidity level was seen to lead to a considerable drop in adult emergence rates.

Results and discussion

Evaluation of all the results had to be made taking into account the interference due to occurring-by-chance negative factors, such as the considerable variability of egg hatching and the sporadic and unforeseeable occurrence of diet contamination. Owing to these factors, comparison of treatments in terms of quantitative results was difficult. It was therefore decided to neither statistically analyze the data nor to represent the results in tables. Nevertheless, as the above-mentioned factors were not directly related to the purpose of our research and as many replicates were made, it was possible to obtain the required information by collecting and integrating data from different replicates. Parasitoid production could thus be evaluated and optimal rearing conditions identified. This procedure was also adopted as the diets employed by us, except those containing low levels of host-derived material, had already proven to be well suited for *E. larvarum* in the previous study (Mellini *et al.*, 1993).

I. Replacement of plastic multiwell plates with glass Petri dishes.

Crowded larvae were seen to be capable of developing without seemingly disturbing each other, even when 72 eggs were originally placed in one Petri dish. In fact, even in this case, about 50 individuals generally completed their larval development so that each larva had only about 0.4 cm^2 of pabulum surface at its disposal.

Besides being more economical, the use of Petri dishes may also result in qualitative improvements of parasitoid production as in our tests mass-reared individuals were seen to grow slightly faster than those reared individually, reach a higher puparium weight and have a more synchronized development.

Moreover, as the mature larvae were incapable of escaping from the Petri dishes, puparia were not dispersed, thus preventing adult waste. In multiwell plates, vice versa, several puparia were lost as quite a few mature larvae abandoned the well or even the plate. In practice, the behaviour pattern of mature larvae grown in plates is similar to that usually exhibited *in vivo*, where the larvae abandon the host and pupate next to host larva remains.

On the other hand, individual rearing in multiwell plates may be advantageous in case of diet contamination by moulds and/or bacteria which remains confined to just a few wells instead of rapidly spreading, as, vice versa, occurs in Petri dishes. This positive feature, however, is not in itself sufficient to offset the above-mentioned disadvantages.

It can be safely concluded that the complex and expensive technique of culturing larvae individually in plastic multiwell plates can be successfully replaced by the easier and more economic mass-rearing in glass Petri dishes.

II. Comparison of agar-containing and cotton-supported diets.

In a preliminary assay, we tested cotton balls of 5 different weights (namely 1.5, 1, 0.75, 0.5, 0.25 g), which were individually placed in 20-cc volume Petri dishes. Best results were obtained when 0.75 and 0.50 g of cotton were used, despite the less-than-full absorption of liquid by the cotton.

When 1.5 g of cotton were used, a part of the cotton remained dry and the Petri dish was too stuffed. When 0.25 g were used, most diet was not absorbed. Larvae were very susceptible to excess liquid and most of them died because they were not capable of preventing their spiracles from being covered by the liquid. In the containers employed by us, therefore, the optimal ratio of absorbent mass to weight of absorbed diet was 3.33-5 g of cotton to 100 g of diet.

Both pupa extract and pupa homogenate were utilized as host-derived material. When cotton was used as a support, the main difference between pupa extract- and pupa homogenate-containing diets was that the former was homogeneously absorbed, whereas the biggest and flocky fragments of the latter were not absorbed and remained on the cotton surface, a situation which, however, did not seemingly affect larval development.

Observations on the egg hatching pattern and behaviour of first- and second-instar larvae were quite easy to conduct on agar and extremely difficult to make on cotton, the surface of which is irregular and opaque. In any case, early-instar larvae were seen to neither sink into the cotton nor to form a sort of respiratory funnel as vice versa they usually do in agar; they actually crawled on the cotton surface and bored into it only upon reaching the third instar.

Independently of the type of host-derived material utilized, puparium production was slightly lower on cotton-supported than on agar-containing media. Moreover, development times were longer on cotton. In case of mass-rearing, however, such disadvantages would be widely compensated for by the fact that cotton, as a diet support, is much cheaper and easier to use than agar. Furthermore, in the two treatments the percent yields of emerged adults were nearly the same. Even with regard to emergence, no appreciable difference was noted between using 0.75 or 0.5 g of cotton.

Absorbent cotton as a physical support for diets was first utilized successfully by Gingrich *et al.* (1971) in the mass-production of the dipteran livestock parasite *Cochliomyia hominivorax* (Coquerel) which was intended for use in sterile male technique programmes. More recently, Bratti and Nettles (1992) utilized cotton-supported diets for the tachinid *Eucelatoria bryani* Sabr. Adult yields were the same as those obtained on agar-containing media.

III. Comparison of pupa extract- and pupa homogenate-containing diets.

Both puparium number and mean weight were higher when non sterilized homogenate was used, independently of the number of eggs, whether one or two, originally placed in a single well.

The mean weight of 92 puparia from pupa homogenate-containing media (= 54.81 mg) was about 35% higher than that of 67 puparia from pupa extract-containing media (= 39.7 mg).

Satisfactory results were also obtained when sterilized pupa homogenate was used, with 2-3 eggs being placed into a single well. The mean weight of 27 puparia (a) obtained from solitary larvae (= 49.27 mg) did not differ from that of 24 puparia (b) obtained from two larvae reared in a single well (= 48.05 mg). This, therefore, demonstrates once again that *E. larvarum* larvae are capable of developing gregariously without seemingly disturbing each other and that the standard amount of diet per well (= 0.625 mg on average) is more than sufficient for two individuals.

In some wells 3 puparia formed, the mean weight of which was considerably less (38.13 mg; n=15). Moreover, most of them did not let the adult emerge, so that the adult yields obtained (13%) were lower than those from puparia (a) and (b), which were equal to 54% and 51% respectively.

In conclusion, replacement of pupa extract with an equal amount of pupa homogenate (better if autoclaved, in order to diminish contamination risks) results in two main advantages. First, diet preparation is much easier and second, the cost of host-derived material is much lower since the amount of pupa extract which can be obtained after centrifuging and filtering is about 1/4 of the original amount of homogenate. Moreover, both the number and the weight of the puparia obtained on pupa homogenate-containing media tend to be higher than those of the puparia obtained on pupa extract-containing diet. This can be ascribed to the fact that while the homogenate is composed of the whole host body, the extract is only made up of a part of it, albeit very important.

The results of the previous paper (Mellini *et al.*, 1993) concerning the replacement of pupa extract with pupa homogenate were therefore confirmed.

IV. Amount of diet fore each larva.

a) Test performed using pupa extract-containing diets set in agar.

Egg hatching variability and diet contamination were higher than usual in this test, which comprised several replicates. In any case, given that a very high number of data was collected and that only non-contaminated diets were considered,

and bearing also in mind the different percentage of hatched eggs, the following conclusions can be drawn.

1) A certain, albeit not strict, relationship exists between the number of eggs placed in rearing containers and the number of puparia obtained. Higher puparium (and adult) yields can therefore be obtained by increasing the number of eggs.

When 24 eggs were originally placed into Petri dishes 12-15 puparia were obtained. This number reached as high as 33-38 when the original number of eggs was 72. Moreover, the developing larvae did not seem to disturb each other either. Unfortunately, no data is available concerning the 96-egg treatment, as massive microbial contamination occurred in all dishes. As a consequence, in such treatment puparium yields were very low.

2) The higher the number of puparia obtained, the lower their weights, though not in proportion. In fact, puparium weight, which on average was of 50-55 mg on an average in the 24-egg treatment, dropped to 40-45 mg in the 72-egg treatment.

3) As a consequence of the above-mentioned pattern, by increasing the original number of eggs placed in diet containers, the parasitoid biomass so highly increased that in the 72-egg treatment it was more than twice as high as in the 24-egg treatment (800 mg versus 1700 mg).

4) Even in the highest crowding condition the conversion index (puparium weight/diet weight) was on average of 0.11-0.12, i.e. three-four times lower than that usually observed in *in vivo* conditions. In one test, the mean weight of 41 puparia obtained on 14 g of diet was 47 mg. The conversion index was therefore about 0.14. As a matter of fact, a considerable amount of diet remained un-used after parasitoid pupation.

5) As a high, though variable, percentage of eggs did not hatch in all of the treatments, the actual amounts of diet per larva were larger than the theoretical ones. For instance, the actual amount of diet was 300 mg in the 72-egg treatment.

6) It can be concluded that higher puparium yields and a more efficient utilization of food on the part of the parasitoid can be obtained with 1 egg per 200 mg of diet. Two to three thousands normal puparia, weighing on average 40 mg, should therefore be obtained by placing 5000 eggs into 1 kg of diet having a surface of 2000 cm^2 and a thickness of 0.5 cm.

7) Higher population density also results in faster larval development rhythms and more uniform puparium weights. Moreover, crowding seemingly limits the spread of microbial contamination at least upon larvae reaching an advanced stage of development.

8) The percent of emerged adults was well over 50 in all of the treatments. Differences among treatments were small. Adult yields did not diminish as the puparium number per container increased.

b) Test performed using pupa homogenate-containing diets set in agar.

The results were similar to those obtained using pupa extract-containing diets. It should however be emphasized once again that homogenate is much cheaper than extract.

V. Attempts to reduce host-derived material in the diet.

a) Tests with pupa extract.

As expected, lower amounts of extract led to worse parasitoid biological parameters, although in different ways.

1) The puparium number per Petri dish dropped from 15-17 in the 20% extract containing diets to 7-9 in the 2.5% containing one. No puparia formed in the diet devoid of host material.

2) The mean weight of puparia dropped from 50 mg in the 20% extract containing diet to 25 mg in the 2.5% extract containing diet. Moreover, as host material decreased, the number of light brown, non-thickened puparia, most of which were anomalous, progressively increased. Larvae fed on the diets containing the lowest amount of extract formed puparia which varied extremely in weight. This pattern proves that different individuals may exhibit different capabilities of adapting themselves to adverse conditions.

3) Owing to the two above-mentioned negative effects, parasitoid biomass considerably diminished, i.e. from 750-850 mg per Petri dish in the 20% extract containing diet to 175-225 mg in the 2.5% extract containing one.

4) In the 20% extract containing diet, at 30°C, development from egg to mature larva required 6-7 days and about 16 days from egg to adult. On the media containing lower quantities of host material, development times were longer. Larval behaviour, on this medium, was different from the one usually exhibited *in vitro*, i.e. larvae did not stop around the entrance hole, but dug long and irregular tunnels into the substrate, thus proving, so to speak, that they did not like this medium.

5) The percentage yields of emerged adults dropped from 60-70% in the standard diet to 10-15% in the diet containing the lowest amount of pupa extract. It should be noted that adult yields did not drop progressively, but were already reduced to 25% in the 10% containing extract.

6) When host material was deleted from the medium, larvae were prevented from reaching the third instar. Before sinking into the diet, first-instar larvae crawled on its surface much longer than they usually do on the standard media. They survived for about 45 days, thus proving that our diet remains physically and chemically suitable for the parasitoid for a long time.

b) Tests with pupa homogenate.

The results were similar to those obtained in the tests employing pupa extract.

In conclusion, as adult yields dropped, even the diet in which host material was reduced just by half was not efficient, notwithstanding the fact that puparium production was quantitatively and qualitatively acceptable. Additional research is needed in order to replace at least a portion of host material with less expensive ingredients.

SUMMARY

The oligidic diet based on bovine serum (75%) and pupa extract (20%) integrated with additives (5%) is very efficient for the *in vitro* rearing of the parasitoid *Exorista larvarum*, thus confirming

the results of a previous study. Although the diet is in itself already very inexpensive as ingredients are quite cheap and preparation is very easy, attempts were made to make it even more economical and efficient in order to permit this parasitoid to be mass-reared for its possible use in IPM programmes against several Lepidoptera, responsible for the defoliation of broadleaf trees.

The study led to the following conclusions:

a) The individual culturing of larvae in plastic multiwell plates can be successfully replaced by mass-rearing in Petri dishes. The latter technique is more economical as glass Petri dishes can be sterilized and re-utilized, whereas plastic multiwell plates are for single use only. Moreover, the complex procedure required for starting the culture is much easier when Petri dishes are employed. Mass-rearing is possible as *E. larvarum* larvae behave gregariously and are capable of developing well even in crowded conditions. Compared to individual culture, mass-rearing may even result in qualitative improvements of the parasitoid production.

b) Agar may be replaced by cotton, used as a physical support for the liquid diet. This replacement is considerably money-saving as, compared to agar, cotton is much cheaper and more practical to use. On cotton, first- and second-instar larvae exhibit a behaviour pattern different from that in the host, but this has no effect on parasitoid mass-rearing.

c) Pupa extract can be replaced by pupa homogenate. The preparation of pupa homogenate is much easier than that of pupa extract, as the latter is obtained by centrifuging the former and passing it through microfilters. Moreover, the use of pupa homogenate permits to save on host-derived material, which vice versa is lost when the extract is prepared.

d) The optimum amount of medium per insect is about 300-350 mg, which leads to appreciable quantitative and qualitative improvements in puparium production. As the larvae maintain an upright position with the posterior pair of spiracles in close contact with air, diet thickness in containers should not exceed 6-7 mm.

e) Host-derived material (both pupa extract and homogenate) is the most expensive ingredient of the diet employed by us. Efforts at reducing it to below 20% were unsuccessful. Puparia formed even when the percent of host material was only 2.5, in which case, however, they were small and, more importantly, did not allow the adult to emerge. Percentages of 5 and 10 led to an appreciable puparium production, but development times were longer and adult yields were still too low. On diets devoid of host material, larvae survived for about 45 days but were not capable of reaching the third instar.

It can be concluded that the results obtained in a- b- c- and d-experiments permitted considerable technical and economical improvements of the *in vitro* rearing of *E. larvarum* to be obtained. Compared to the original standard diet and notwithstanding the simplier techniques employed, adult yields remained very high and some of the biological parameters of the parasitoid were even seen to improve.

Miglioramenti tecnici nell'allevamento del parassitoide *Exorista larvarum* L. (Dipt. Tachinidae) su dieta oligidica.

RIASSUNTO

La dieta oligidica a base di siero bovino (75%) e di estratto di crisalide (20%) con l'aggiunta di alcuni additivi (5%) si è confermata quale ottimo substrato per l'allevamento del parassitoide *Exorista larvarum*. Pur essendo molto economica, per il tipo di ingredienti e la semplicità di preparazione, si sono svolte ulteriori indagini per migliorare queste caratteristiche, in vista di allevamenti massali per il possibile impiego del tachinide in programmi di lotta biologica contro alcuni lepidotteri fillofagi di latifoglie. La presente sperimentazione ha dimostrato quanto segue.

a) È possibile sostituire le piastre multicellari in plastica, per l'allevamento di singole larve, con capsule Petri in vetro per l'allevamento collettivo. In tal modo si realizza un forte risparmio in riguardo ai contenitori, essendo le capsule, a differenza delle piastre, riciclabili a piacere. Inoltre restano notevolmente semplificate le operazioni tecniche iniziali. Tutto ciò è possibile perchè il tachinide è un parassitoide gregario che si è dimostrato capace di svilupparsi in modo ottimale anche in condizioni di forte affollamento: in esse tende addirittura a migliorare alcuni parametri biologici rispetto alla condizione solitaria.

b) È possibile sostituire l'agar con cotone idrofilo, come supporto fisico della dieta liquida. Anche con questa operazione i vantaggi sono notevoli in termini di economicità, sia per il bassissimo costo del cotone in confronto all'agar, sia per l'estrema facilità del suo impiego. Il comportamento delle larve di I e II età nella dieta con cotone si discosta, a differenza di quanto accade in pabulum agarizzato, da quello nell'ospite, ma ciò appare ininfluente agli effetti di un allevamento massale.

c) È possibile sostituire l'estratto di crisalide con un omogeneizzato in toto delle medesime. La preparazione dell'omogeneizzato è assai più semplice di quella dell'estratto, che è ottenuto per centrifugazione e microfiltrazione del primo. Inoltre non comporta una cospicua perdita del materiale proveniente dall'ospite, come accade invece per l'allestimento dell'estratto.

d) Il quantitativo ottimale di dieta pro capite può essere indicato intorno ai 300-350 mg. Tale dose garantisce una buona produzione sia quantitativa che qualitativa di pupari. Viste le modalità di vita delle larve, che stazionano in posizione verticale e mantengono gli stigmi posteriori in comunicazione con l'aria, è opportuno che lo spessore del pabulum nei contenitori non superi, per un suo migliore sfruttamento, i 6-7 mm.

e) I tentativi per ridurre l'aliquota di materiale proveniente dall'ospite, sia esso sotto forma di estratto od omogeneizzato di crisalidi, hanno invece dato risultati insoddisfacenti. Anche con solo il 2,5% si formano pupari, ma pochi, di basso peso e per di più incapaci di dare gli adulti. Con dosi più elevate la produzione dei pupari, anche se ritardata, è buona, ma gli sfarfallamenti si attestano su livelli troppo bassi. Pertanto il problema di diminuire dall'attuale 20% la percentuale di componenti dell'ospite, che è l'ingrediente più costoso, rimane aperto. In completa assenza di tale materiale, le larvette sopravvivono per oltre un mese senza però oltrepassare il II stadio.

In conclusione, la sperimentazione di cui ai punti a-d ha consentito un notevole miglioramento, sia dal punto di vista tecnico che economico, dell'allevamento *in vitro* di *E. larvarum*. Rispetto alla validissima dieta standard iniziale, non solo viene mantenuto, nonostante la semplificazione, l'elevato livello produttivo, ma addirittura si sono avuti miglioramenti in riguardo ad alcuni parametri biologici del parassitoide.

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